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TITLE: p19 ARF-p53 Tumor Suppressor Pathway During Oncogene-
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PRINCIPAL INVESTIGATOR: Jianping Jin, Ph.D.
Dr. Scott W. Lowe

CONTRACTING ORGANIZATION: Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

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6. AUTHOR(S)
Jianping Jin, Ph.D.
Dr. Scott W. Lowe

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

E-Mail: jin@cshl.org

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13. ABSTRACT (Maximum 200 Words)

The primary objective of this study is to provide new insights into the role of the p53 and ARF tumor suppressors in cancer development and therapy. During the second year of this project, we found that E1A oncogene can increase mRNA levels of tumor suppressor ARF. The region of E1A responsible for ARF induction is also important for apoptosis function of E1A. We also found that oncogene E1A can reduce apoptosis inhibitor gene, A20, expression, which can inhibit E1A-induced apoptosis once overexpressed. In collaboration with C. Sher, we tried to produce different monoclonal antibodies to p19ARF. Right now, we are doing the intensive screens to determine the antibodies specificity.

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Introduction

Cancer is characterized by dramatic changes in the abundance of proteins that promote growth and metastasis. One of the major changes in tumorigenesis is the loss of the function of tumor suppressor genes. ARF-p53 tumor surveillance pathway has been shown to play important roles in tumor suppression. Mutations in the p53 and other tumor suppressor genes are the most common genetic changes found in breast cancer so far. We and others found that oncogenes induce p53 through a signal transduction pathway that requires the presence of another tumor suppressor, ARF. This pathway, when activated, directs the cells to apoptosis or cell cycle arrest which then prevent tumor cell development. The primary objective of this project is to provide new insights into the action of the tumor suppressor p53 and ARF and the regulation of these two tumor suppressors in cancer development. Our original studies took advantage of mouse cells with a defined genetic background to analyze the relevance of single genes in the oncogene-p19^{ARF}-p53 pathway. We proposed to compare this highly controlled system to normal human breast cells in order to determine if the pathway is conserved in human cells and thus potentially relevant to breast cancer. Our early study also indicated that oncogene E1A can sensitize the apoptosis of primary cells which is directly relative to ARF/p53 pathway. Furthermore, by comparing and contrasting normal versus oncogene expressing cells, in these systems, we will be able to get insight to the mechanisms by which p19^{ARF} is involved in p53 activation. Finally, a better understanding of the oncogene-p19^{ARF}-p53-apoptosis pathway could allow us to exploit new strategies to enhance the chemo- and radio-sensitivity of breast cancer cells. The research progress of the second year on this project is reported as follows.

Body

1. Oncogene signaling to p53 (Aims 2 and 3)

The primary objective of this study is to provide new insights into the role of the p53 and ARF tumor suppressors in breast cancer development and therapy. p53 promotes cell cycle arrest or apoptosis in response to different stimuli, including DNA damage and

mitogenic stimulation. Following DNA damage, signaling is mediated, at least in part, by a kinase that phosphorylates p53 on Serine 15, thereby stabilizing the protein by preventing its association with its negative regulator Mdm2.

In our original proposal, we presented data showing that the adenovirus E1A oncogene activates p53 through a mechanism distinct from DNA damage, involving cancellation of Rb function and activation of the ARF tumor suppressor, leading the cells to apoptosis. ARF-null MEFs expressing E1A are incapable of activating the p53 response and are more resistant to apoptosis following serum depletion or adriamycin treatment, compared to wild type MEFs. Reintroduction of ARF restores p53 accumulation and sensitizes cells to radiation and chemotherapy.

During the second year of this research, we decided to test whether ARF induction is a common event following oncogene overexpression. We found that E1A can induce ARF expression both in human and mouse primary cells. More importantly, northern blot indicated that E1A-induced ARF expression results from the increase of ARF mRNA levels.

Our published and unpublished data indicated that different regions of E1A correspond to various function of E1A. For example, the CR2 region has been shown to bind to RB and be essential to apoptosis function of E1A (Samuelson et al., 1997). We also found that p300 binding region which locates at N-terminal part of E1A is not important to the apoptosis function of E1A. However, region 26-35 is important. Now, we are focusing on which regions of E1A polypeptide are responsible for the increase of ARF mRNA levels by E1A in primary cells. We expected that the regions important for E1A's apoptosis function should be also important for ARF induction. Our preliminary data suggested that both CR2 and 26-35 regions are important for the increase of ARF mRNA levels. In collaboration with Dr. David Livingston's Lab at Harvard University, we found that the 26-35 region binds p400/TRAPP complex which possibly involved in ARF induction at transcription levels (Samuelson et al., unpublished). Next, we will focus on 26-35 region for ARF induction. Our data indicated that region 26-35 does not completely lose the function of E1A in apoptosis induction. Therefore, we will make more mutants around this region to study ARF induction more deeply.

2. Production of highly specific monoclonal antibodies against the N-terminal portion of ARF(Aim 1)

In human cancers, ARF mutations occur mainly in the region coding for the C-terminus domain (exon 2), possibly leading to the formation of truncated isoforms. These putative forms would be impossible to be detected by the currently available anti-ARF antibodies, which are directed against the C-terminus of the protein. In the original proposal, we proposed to raise antibodies against the N-terminal region of ARF, which has been shown to be necessary and sufficient to induce cell cycle arrest and to interact with p53 and/or Mdm2.

In collaboration with C. Sherr, we produced and analyzed different monoclonal antibodies. The CSHL Monoclonal Service Facility performed all the animal work, the fusions, single cell cloning and initial screens. We am currently doing the extensive screens to determine the antibodies specificity. If we found the specific antibodies for ARF, we will initially use them for routine characterization studies, such as western blot analysis and immunofluorescence, to detect ARF levels in different cell lines and to investigate the presence and abundance of the truncated isoforms in tumor derived cell lines and in clinical specimens.

3. The function of E1A in suppression of anti-apoptosis proteins

Beside the induction of positive apoptosis effector by oncogenes, I am also interested in the regulation of apoptosis inhibitors by oncogenes. We found that E1A can repress A20, an apoptosis inhibitor, using a Clontech Atlas filter array. Since A20 has been shown to be overexpressed in undifferentiated nasopharyngeal carcinoma, head & neck squamous cell carcinomas, it would be interesting to see whether A20 could be one of the reasons that many cancers are resistant to cancer drug treatments and whether A20 would be a good drug target for the cancers with high A20 expression. I confirmed that repression of A20 by E1A exists generally in human and mouse primary cells by northern blot. Interestingly, it is possible that E1A represses A20 expression by affecting A20 mRNA stability. Now, I have started to determine whether other oncogenes could have similar function as E1A in inhibition of A20. I also made A20/retrovirus by retrovirus system, I infected the mammalian primary cells with these A20/retrovirus, I was able to

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show that A20 overexpression could block oncogene/cancer drug-induced apoptosis in some cells.

Key Research Accomplishments

We found that E1A can induce ARF expression by transcription and/or post-transcription regulation. Consequently, The function of Mdm2 is inhibited and tumor suppress p53 be stabilized by elevated ARF protein levels. More importantly, this induction of ARF by E1A is correlated to apoptosis induction by E1A in primary cells. The region from residue 26 to 35 and conserved region 2 (CR2) of E1A 12S polypeptide is important to apoptosis function of E1A. Our data indicated that these two regions are responsible for ARF induction by E1A at mRNA levels.

We also examined the role of oncogene E1A in regulation of an apoptosis inhibitor, A20. We found oncogene E1A can inhibit A20 expression at mRNA level. Most interesting, overexpression of A20 can block E1A/cancer drug-induced apoptosis.

In collaboration with C. Sherr, we tried to produce and analyze different monoclonal antibodies to p19^{ARF}. Right now, we are in the process of intensive screens to determine the antibodies specificity.

Reportable Outcomes

The research is still ongoing. In collaboration with Mr. Samuelson, we will have a paper about E1A-induced ARF expression in a shot time. The paper is in writing. At this moment, there is no reportable outcome.

Conclusions

In conclusion, oncogenes can stabilize p53 level by activating ARF at mRNA levels. In collaboration with Mr. Samuelson, I found that oncogene E1A induces ARF mRNA

expression. Mutational analysis indicated that the regions of E1A polypeptide responsible for ARF induction are also important to apoptosis function of E1A. These data supplied the solid support for ARF-p53 pathway in tumor surveillance. How E1A induce ARF expression is under investigation in our Laboratory. In collaboration with Dr. de Stanchina, I also E1A can repress the expression of an apoptosis inhibitor, A20, beside activate ARF-p53 pathway. Our preliminary data indicated that the repression of A20 by oncogene E1A could be a general phenomena. Oncogenes, E2F and myc may have similar function. We are continuing to work on this project, with a particular emphasis on how E1A induces ARF expression.

References

N/A

Appendices

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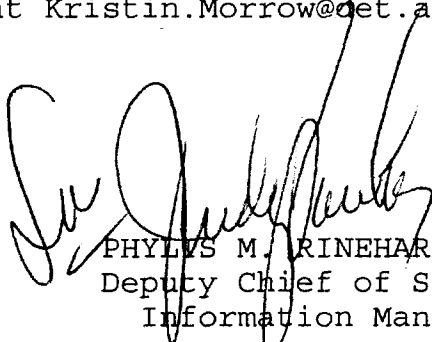VA 22060-6218

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